

# Emissions of nitrous acid (HONO), nitric oxide (NO), and nitrous oxide (N<sub>2</sub>O) from horse dung

Marja Maljanen, Zafar Gondal, Hem Raj Bhattarai

University of Eastern Finland, Department of Environmental and Biological Sciences, P.O. Box 1627, 70211 Kuopio  
email: Marja.Maljanen@uef.fi

Horse dung contains considerable amounts of nitrogen which is partly lost during the storage period. Leaching of nitrogen from the dung can be prevented with constructions but also gaseous N-emissions occur. However, the emission rates are not reported in the literature. We measured in laboratory conditions nitrous oxide (N<sub>2</sub>O), nitric oxide (NO) and nitrous acid (HONO) emissions from fresh, one month old and one year old horse dung samples. NO and HONO emissions increased with the storage time of the dung. The mean emission rates of HONO and NO were from 36 to 280 ng N kg dw<sup>-1</sup> h<sup>-1</sup> and from 15 to 3500 ng N kg dw<sup>-1</sup> h<sup>-1</sup>, respectively. N<sub>2</sub>O emissions were more variable showing also highest emissions (20.3 µg N kg dw<sup>-1</sup> h<sup>-1</sup>) from the oldest samples. Thus, the longer storage of horse dung increases gaseous N losses which should be taken into account when planning an environmental friendly way to handle horse dung.

*Key words:* nitrogen, carbon, nitrification, greenhouse gas, animal excreta

## Introduction

The number of horses is increasing in the EU countries including Finland. There are currently 75 000 horses in Finland and these horses are producing more than one million m<sup>3</sup> manure annually, of which a small portion is left on the pastures during the summer (Manninen et al. 2016). Annually about 770 000 m<sup>3</sup> of manure is collected from the stables, and it is mainly used as fertilizer in the fields. However, at the moment most of the new stables are established in the urban areas/without connection to agricultural fields, which causes problems with the manure management and the cost for disposing of the manure is significant (Manninen et al. 2016).

At horse stables manure is stored in stockpiles on e.g. concrete bottom structure or in a skip before transportation or further processing. There are requirements for the stockpiling the manure to prevent leaching of nutrients, e.g. nitrate, by Government Decree on the Restriction of Discharge of Nitrates From Agriculture into Waters based on the EU Nitrates Directive (<http://www.finlex.fi/en/laki/kaannokset/2000/en20000931.pdf>). However, during this storage period also the gaseous N-losses e.g. ammonia (NH<sub>3</sub>) or nitrous oxide (N<sub>2</sub>O) from the manure are taking place (Thomsen 2000, Garlipp et al. 2011). Emissions from manure may vary with storage time and moisture (Pratt et al. 2015) and depend also of the bedding material (e.g. straw, wood chips, peat) used (Garlipp et al. 2011). However, these direct emission rates from horse manure or dung itself are not reported in the literature.

Nitrous oxide (N<sub>2</sub>O) is a strong greenhouse gas (about 300 times stronger than CO<sub>2</sub>) mainly originating from agriculture and livestock manure management accounts for almost 10% of greenhouse gas emissions from agriculture globally (Owen and Silver 2015). Another N-gas which is produced in the same microbial processes as N<sub>2</sub>O is nitric oxide (NO). NO is not a greenhouse gas but it reacts to form smog and acid rain and it is also important to the formation of tropospheric ozone (Heil et al. 2016). Nitrous acid (HONO), also a reactive gas in the atmosphere, is linked to nitrogen cycle processes and thus also the production of N<sub>2</sub>O and NO (Su et al. 2011, Maljanen et al. 2013). It is not a greenhouse gas, instead, it contributes to formation of hydroxyl radicals (OH<sup>-</sup>), which are strong oxidizing molecules and can oxidize e.g. atmospheric methane (CH<sub>4</sub>) (Riedel and Lassey 2008). Formation of aerosols (e.g. sulfuric acid and volatile organic compounds), can speed up the HONO emissions, which are linked to air pollution and climate change (Kulmala and Petäjä 2011). The soil related sources and formation pathways of HONO are not well known. HONO is produced in the atmosphere by photolytic reaction of nitrite (NO<sub>2</sub><sup>-</sup>) or humic acid with nitrogen dioxide (Stemmler et al. 2006) but also in soil processes in N-rich soils (Su et al. 2011, Maljanen et al. 2013, Oswald et al. 2013, Scharcko et al. 2015). Large HONO emissions have been reported from N-rich organic soils with low C/N ratio (Maljanen et al. 2013) and our hypothesis is that animal dung with high organic matter and N-content can also emit significant amount of HONO as well as the other N-gases; NO and N<sub>2</sub>O.

To our knowledge there are no published studies on the gaseous N emissions (e.g.  $N_2O$ , NO or HONO) from horse dung but several research papers report  $N_2O$  emissions from cattle, sheep or poultry manure (e.g. Larios et al. 2016). Since horse dung has different composition and structure than e.g. dairy cow dung, the gaseous emissions may differ significantly. These emissions should be known when planning the environmental friendly way to handle the horse dung. To study the effect of storage time we measured these gaseous N-emissions from fresh, short term stored and long term stored horse dung.

## Materials and methods

The horse dung was sampled in February 2016 from a stable located in Eastern Finland (mean annual T 3.2. °C, annual precipitation 630 mm, Pirinen et al. 2012). The stable is housing about 40 horses and the diet consists mainly oats and hay. Fresh dung (F) samples (< 12 h) were collected directly from several boxes inside the stable, short term stored (about 1 month, M) and older samples (stored at least one year, Y) were collected from several sampling points from outdoor manure storage piles on concrete floor which were covered with a roof. Each type of sample was first collected in a 20 l plastic bucket, covered with a lid and transported to laboratory within 2 hours. The bedding material used in the stable is saw dust pellets. Dung was sampled as free from bedding material as possible. Before manual mixing and splitting into sub samples the remaining bedding material was removed manually. Four replicate sub samples (~150 g dw) were placed in PVC rings (diameter 18 cm) which were sealed from the bottom with an aluminium foil. Rings were incubated over night at room temperature (20 °C) before gas flux measurements. Gas fluxes ( $N_2O$ , NO and HONO) were measured first at original moisture content (Table 1) and after adjusting all samples to same moisture level (2 g  $H_2O$  g  $dw^{-1}$ ) and incubation of 13 days. For adjusting the moisture the F and M samples were let to dry at room temperature whereas milliQ  $H_2O$  was added in the Y samples to increase moisture content. However, water was evaporating faster from the Y samples during the gas flux measurements and the final moisture of Y samples was therefore less than the targeted value.

pH and electrical conductivity (EC) were measured from sample:water slurry (30:50 v/v). Total C and N were analysed with vario MAX elemental analyser (Elementar Analysensysteme GmbH, Germany). For analysis of nitrate ( $NO_3^-$ ), nitrite ( $NO_2^-$ ) and dissolved organic carbon (DOC) a 30 ml sample and 100 ml milliQ- $H_2O$  were shaken at 175 rpm for one hour and then filtered and analyzed with an ion chromatograph (DX 120, Dionex Corporation, USA) for  $NO_3^-$  and  $NO_2^-$  and with Shimadzu TOC-VCPH/CPN analyzer (Shimadzu, Japan) for DOC. To extract ammonium ( $NH_4^+$ ) a sample of 30 ml dung and 100 ml 1M KCl were used and  $NH_4^+$  was analyzed with spectrophotometer. Gravimetric moisture was determined by drying the samples for 24 h at 65 °C. Organic matter (OM) concentration was determined by loss on ignition at 550 °C (e.g. Maljanen et al. 2013).

HONO emissions were measured by dynamic flow chamber connected with a commercial HONO analyzer (LOPAP, QUMA Elektronik & Analytik GmbH, Germany), see supplementary information for details. NO fluxes were measured by dynamic chamber connected with Thermo 42i NOx analyzer (Thermo Fisher Scientific).  $N_2O$  fluxes were measured with a static chamber system and samples were taken with syringes from the headspace of the chamber at intervals of 5, 10, 15 and 20 min after closing the chamber. Gas samples were injected into 12 ml Exetainers (Labco, UK) and were analyzed with a gas chromatograph (Agilent 7890B, Agilent Technologies, USA). In addition to  $N_2O$  also  $CH_4$  and  $CO_2$  fluxes at initial moisture content were measured with the same method. Statistical differences in the N-gas fluxes were tested with non-parametric Mann Whitney U-test since the data was not normally distributed. Gas fluxes before and after adjusting moisture were treated separately. Other parameters were analyzed by One-Way ANOVA and Tukey's test.

## Results

OM and C concentration and C/N ratio were significantly lower in Y samples than in the other samples (Table 1). DOC concentration decreased with increasing age of dung. The initial moisture content was higher in F and M samples than in Y sample, as expected. All dung samples had pH above 7 and EC above 800  $\mu S\ cm^{-1}$ .

All studied horse dung samples emitted both HONO and NO, but only F and Y samples emitted  $N_2O$ . Storage time of dung increased clearly the emissions of NO and HONO and there was a clear statistical difference between F, M and Y samples (Fig. 1). The emission rates of NO were  $56 \pm 22$ ,  $900 \pm 77$  and  $3000 \pm 670$  and emission rates of HONO were  $35 \pm 19$ ,  $53 \pm 20$  and  $113 \pm 34\ ng\ N\ kg\ dw^{-1}\ h^{-1}$  in F, M and Y samples at original moisture content, respectively (Fig. 1).

Table 1. Sample properties (average ± standard deviation) measured from four replicate dung samples. EC = electrical conductivity ( $\mu\text{S cm}^{-1}$ ), OM = organic matter concentration (% of dw), C = total carbon concentration (% of dw), N = total nitrogen concentration (% of dw), C/N = carbon to nitrogen ratio, DOC = water extractable dissolved organic carbon ( $\text{mg g dw}^{-1}$ ), GM = initial and adjusted gravimetric moisture ( $\text{g H}_2\text{O g dw}^{-1}$ ),  $\text{CH}_4$  = methane production rate ( $\mu\text{g g dw}^{-1} \text{h}^{-1}$ ) and  $\text{CO}_2$  = carbon dioxide production rate ( $\text{mg g dw}^{-1} \text{h}^{-1}$ ) measured at initial moisture.

	Fresh (F)	One month old (M)	One year old (Y)	F-value
pH	7.5	7.9	7.7	1.64
EC	$810 \pm 180^a$	$1670 \pm 330^b$	$1630 \pm 350^b$	10.43**
OM	$90.1 \pm 0.4^a$	$90.6 \pm 1.0^a$	$65.7 \pm 8.1^b$	36.42**
C	$44 \pm 0.1^a$	$43 \pm 0.4^a$	$31.8 \pm 4.6^b$	27.31**
N	$1.8 \pm 0.1$	$1.6 \pm 0.1$	$1.8 \pm 0.3$	1.07
C/N	$25.0 \pm 0.7^a$	$27.6 \pm 0.8^b$	$18.1 \pm 1.5^c$	83.61**
DOC	$18.8 \pm 0.6^a$	$13.5 \pm 0.9^b$	$11.3 \pm 1.8^b$	97.43**
GM <sub>initial</sub>	$3.63 \pm 0.12^a$	$3.05 \pm 0.15^a$	$0.56 \pm 0.13^b$	12.29**
GM <sub>adjusted</sub>	$1.98 \pm 0.10$	$1.94 \pm 0.05$	$1.58 \pm 0.40$	1.85
$\text{CH}_4$	$20.8 \pm 4.1^a$	$10.0 \pm 3.3^b$	$0.61 \pm 2.4^c$	36.76**
$\text{CO}_2$	$1140 \pm 280^a$	$1940 \pm 400^a$	$120 \pm 54^b$	41.61**

The statistical difference between sample types were analyzed by One-Way ANOVA; \*\* =  $p < 0.01$ . Values with a common letter as superscript do not differ as a statistical significance of  $p < 0.05$  in Tukey's test.

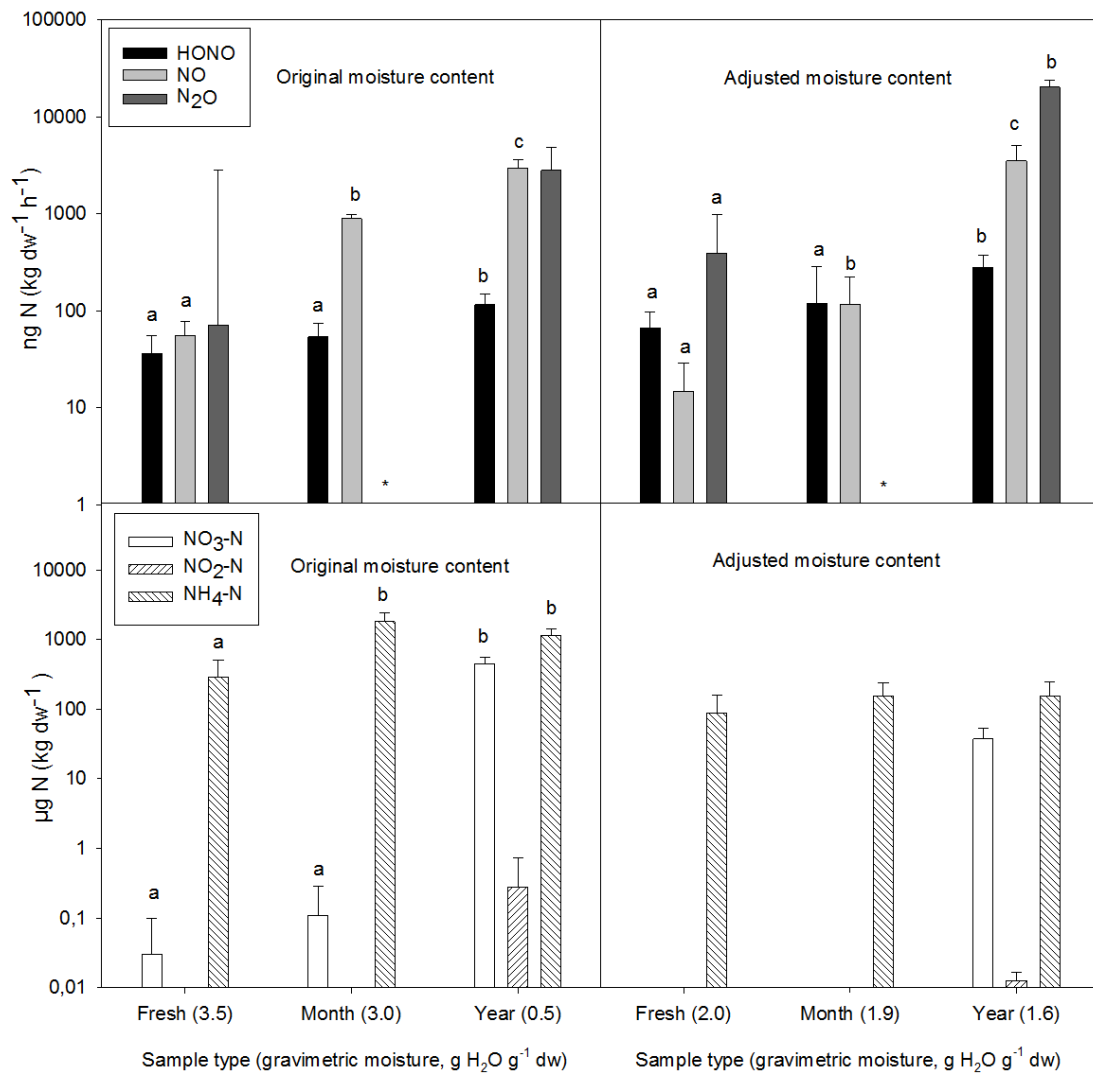


Fig. 1. HONO, NO and N<sub>2</sub>O emissions and mineral nitrogen concentrations from samples in a) original moisture content and b) adjusted moisture content. \*) The average N<sub>2</sub>O emission from M sample was negative (mean N<sub>2</sub>O consumption rate  $510 \pm 330$  and  $350 \pm 280$  ng N kg dw<sup>-1</sup> at original and adjusted moisture) and therefore it is not shown in the figure with a log-scale on y-axis. Different letters indicate statistical difference between sample types (Mann Whitney U-test).

HONO emissions were five times and NO emissions 60 times higher in one year old sample than in fresh dung at original moisture level but 30 and 500 times higher after adjusting the moisture, respectively. N<sub>2</sub>O emissions were more variable but also the highest N<sub>2</sub>O emissions ( $20.3 \pm 2 \mu\text{g N kg dw}^{-1} \text{ h}^{-1}$ ) were measured from the oldest samples after adjusting the moisture (Fig. 1). F and Y samples emitted N<sub>2</sub>O but M samples had negative mean N<sub>2</sub>O emission (uptake). The mean N<sub>2</sub>O uptake rate in M samples was  $510 \pm 333$  and  $353 \pm 277 \text{ ng N kg dw}^{-1}$  at original and adjusted moisture levels, respectively (not shown in Fig. 1 with log-scale on y-axis). The mean N<sub>2</sub>O emission from Y samples were from 40 to 50 times higher than those from F samples.

NO<sub>3</sub><sup>-</sup> concentration in dung increased with age of the manure at initial moisture content (Fig. 1). After adjusting the moisture content and incubation of 13 days concentrations were lower than the detection limit ( $0.01 \mu\text{g g}^{-1}$ ) in F and M samples. NO<sub>2</sub><sup>-</sup> was detectable ( $> 0.01 \mu\text{g g}^{-1}$ ) only from Y samples (Fig. 1). Ammonium concentration was lower in F samples than in M or Y at original moisture content but did not differ significantly after adjusting the moisture.

Methane emissions at initial moisture content decreased with dung age (Table 1). Some of the Y samples even consumed CH<sub>4</sub>. CO<sub>2</sub> emissions from the dung were significantly higher from younger F and M samples than from Y samples (Table 1).

## Discussion

All dung sample types emitted NO and HONO and emission rates increased with storage time. Also N<sub>2</sub>O was emitted from F and Y samples, but surprisingly not from M samples where net N<sub>2</sub>O uptake was measured. The reason for low N<sub>2</sub>O emission from M samples is not clear, M samples were collected in winter and perhaps the storage of dung at low temperature (from 2 to  $-15 \text{ }^\circ\text{C}$ ) had decreased nitrogen mineralization. We assume that higher emissions from Y samples is a result of increased N mineralization/ nitrification rate during long term storage. When manure is stored, a portion of the organic N is converted by soil microbes to NH<sub>4</sub>. NH<sub>4</sub> is then oxidized by different microbes to nitrate and then converted to gaseous N (including N<sub>2</sub>, N<sub>2</sub>O, NO and HONO) (Oswald et al. 2013, Owen and Silver 2015, Scharko et al. 2015). However, in storage piles dung is mixed with urine and beddings which can also affect the amount of available N. Ammonium was not accumulating in the dung samples indicating that it was used rapidly in nitrification. Detectable amounts of NO<sub>2</sub><sup>-</sup> was found only from Y dung, this could be linked to higher HONO emissions which can be formed chemically from NO<sub>2</sub><sup>-</sup> in acidic conditions (Su et al. 2011). Since the pH of horse dung is neutral or alkaline the microbial pathway (mainly via nitrifiers) of HONO is also possible. The C/N ratio in our study was between 18 and 27, which is slightly lower than reported by Swinker et al. (1997) for horse manure but optimal for microbial processes. Thus, gaseous N emissions from the horse dung can be linked with both physio-chemical (Su et al. 2011, Heil et al. 2016) and microbial processes (Oswald et al. 2013, Scharko et al. 2015).

There are no published results about direct GHG emissions from horse dung. Some studies (Garlipp et al. 2011, Borhan et al. 2014) are showing that horse dung is emitting N<sub>2</sub>O and CH<sub>4</sub> but actual flux rates are not reported. In some reports reference N<sub>2</sub>O emission factor of 0.25% of total N concentration have been used (e.g. Manninen et al. 2016). If we use the N<sub>2</sub>O emissions rate from Y samples, it gives 0.038 g of N<sub>2</sub>O yr<sup>-1</sup> from 1 kg of dung, corresponding 0.22% of total N calculated for one year, which is close to the reported emission factor.

If we compare the highest mean Y horse dung emissions calculated per area ( $182 \mu\text{g N}_2\text{O-N m}^{-2} \text{ h}^{-1}$  and  $52 \mu\text{g NO-N m}^{-2} \text{ h}^{-1}$ ) with those from dairy cow dung patches measured with similar chamber and analyzer systems in the field in Eastern Finland ( $110 \pm 20 \mu\text{g N}_2\text{O-N m}^{-2} \text{ h}^{-1}$  and  $15 \pm 2 \mu\text{g NO-N m}^{-2} \text{ h}^{-1}$ ) we can see that NO and N<sub>2</sub>O emissions from horse dung are higher than the mean emissions from dairy cow dung (Maljanen et al. 2007). However, the methane emissions from horse dung are lower than those from dairy cow dung (Maljanen et al. 2012) as a result of lower moisture content and differences in digestion (ruminant – non ruminant). Methane emissions decreased with the age of the dung because CH<sub>4</sub> from fresh dung originates from the anaerobic conditions in the digestion and no CH<sub>4</sub> is produced in aerobic conditions. CO<sub>2</sub> production rate was lowest in the oldest sample and highest in M samples which could be associated to fast decomposition of M samples at room temperature after storage below 0 °C temperatures.

Our study was made in laboratory conditions in constant temperature and humidity. Field measurements from stockpiles or from croplands where dung is spread as fertilizer would be necessary to estimate the real N-gas field emissions. It seems that horse dung is very potential source of N-gases, including still rather unknown HONO gas. The mean HONO-N emissions were only about 4% of the mean N<sub>2</sub>O-N emissions and therefore the positive climate effect of HONO cannot compensate the warming effect of N<sub>2</sub>O from horse dung. However, the HONO emissions measured here from the one Y dung samples were among the highest “soil” related HONO sources published so far (Su et al. 2011, Maljanen et al. 2013).

This study shows that nitrogen is lost from horse dung in addition to ammonia also in forms of HONO, NO and N<sub>2</sub>O. The storage of horse dung enhances gaseous N losses which should be taken into account in life cycle analysis and when planning an environmental friendly way to handle horse dung.

### Acknowledgements

The study was funded by Marjatta and Eino Kolli foundation. We thank all the horses at Tapsan Talli Ranta-Toivala, Finland for providing material for the study.

### References

- Borhan, S., Rahman, S. & Hammer, C. 2014. Water Absorption Capacity of Flax and Pine Horse Beddings and Gaseous Concentrations in Bedded Stalls. *Journal of Equine Veterinary Science* 34: 611–618.
- Garlipp, F., Hessen, E.F & van den Weghe, H.F.A. 2011. Characteristics of gas generation (NH<sub>3</sub>, CH<sub>4</sub>, N<sub>2</sub>O, CO<sub>2</sub>, H<sub>2</sub>O) from horse manure added to different bedding materials used in deep litter bedding systems. *Journal of Equine Veterinary Science* 31: 383–395. <http://www.finlex.fi/en/laki/kaannokset/2000/en20000931.pdf>. Government Decree on the Restriction of Discharge of Nitrates From Agriculture into Waters.
- Heil, J., Vereecken, H. & Brüggemann, N. 2016. A review of chemical reactions of nitrification intermediates and their role in nitrogen cycling and nitrogen trace gas formation in soil. *European Journal of Soil Science* 67: 23–39.
- Kulmala, M. & Petäjä, T. 2011. Soil Nitrites Influence Atmospheric Chemistry. *Science* 333: 1586–1587.
- Larios, A.D., Brar, S.K., Ramirez, A.A., Godbout, S., Sandoval-Salas, F. & Palacios, J.H. 2016. Challenges in the measurement of emissions of nitrous oxide and methane from livestock sector. *Reviews of Environmental Science and Biotechnology* 15: 285–297.
- Manninen, K., Grönroos, J., Luostarinen, S. & Saastamoinen, M. 2016. Hevosennälän energiakäytön ympäristövaikutukset. Luonnonvara- ja biotalouden tutkimus 30/2016. Luke Luonnonvarakeskus. (in Finnish).
- Maljanen M., Martikkala, M., Koponen, H.T., Virkajärvi, P. & Martikainen, P.J. 2007. Fluxes of nitrous oxide and nitric oxide from experimental excreta patches in boreal agricultural soil. *Soil Biology and Biochemistry* 39: 914–920.
- Maljanen, M., Virkajärvi, P. & Martikainen, P.J. 2012. Dairy cow excreta patches change the boreal grass swards from sink to source of methane. *Agricultural and Food Science* 21: 91–99.
- Maljanen, M., Yli-Pirilä, P., Hytönen, J., Joutsensaari, J. & Martikainen, P.J. 2013. Acidic northern soils as sources of atmospheric nitrous acid (HONO). *Soil Biology and Biochemistry* 67: 94–97.
- Oswald, R., Behrendt, T., Ermel, M., Wu, D., Su, H., Cheng, Y., Breuninger, C., Moravek, A., Mouglin, E., Delon, C., Loubet, B., Pommerening-Röser, A., Sörgel, M., Pöschl, U., Hoffmann, T., Andreae, M.O., Meixner, F.X. & Trebs, I. 2013. HONO Emissions from Soil Bacteria as a Major Source of Atmospheric Reactive Nitrogen. *Science* 341: 1233–1235.
- Owen, J.J. & Silver, W.L. 2015. Greenhouse gas emissions from dairy manure management: a review of field-based studies. *Global Change Biology* 21: 550–565.
- Pirinen, P., Simola, H., Aalto, J., Kaukoranta, J.-P., Karlsson, P. & Ruuhela, R. 2012. Climatological statistics of Finland 1981-2010. Reports 2012:1. Finnish Meteorological Institute, Helsinki.
- Pratt, C., Redding, M., Hill, J. & Jensen, P.D. 2015. Does manure management affect the latent greenhouse gas emitting potential of livestock manures? *Waste management* 46: 568–576.
- Riedel, K. & Lassey, K. 2008. Detergent of the atmosphere. *Water & Atmosphere* 16: 22–23.
- Scharko, N.K., Schütte, U.M.E., Berke, A.E., Banina, L., Peel, H.R., Donaldson, M.A., Hemmerich, C., White, J.R. & Raff, J.D. 2015. Combined Flux Chamber and Genomics Approach Links Nitrous Acid Emissions to Ammonia Oxidizing Bacteria and Archaea in Urban and Agricultural Soil. *Environmental Science and Technology* 49: 13825–13834.
- Stemmler, K., Ammann, M., Donders, C., Kleffmann, J. & George, C. 2006. Photosensitized reduction of nitrogen dioxide on humic acid as a source of nitrous acid. *Nature* 440: 195–198.
- Su, H., Cheng, Y., Oswald, R., Behrendt, T., Trebs, I., Meixner, F., Andreae, M.O., Cheng, P., Zhang Y. & Pöschl, U. 2011. Soil nitrite as a source of atmospheric HONO and OH radicals. *Science* 333: 1616–1618.
- Swinker, A.M., Tanner, M.K., Johnson, D.E. & Benner, L. 1997. Composting characteristics of three bedding materials. *Journal of Equine Veterinary Science* 18: 462–466.
- Thomsen, I.K. 2000. C and N transformations in <sup>15</sup>N cross-labelled solid ruminant manure during anaerobic and aerobic storage. *Bioresource Technology* 72: 267–274.